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GENETIC FEATURES OF STREPTOCOCCUS PNEUMONIAE AND ITS ROLE IN CAUSING MENINGITIS

Dunya Talib Mahdi 1, Rana Jaafar Abed 2
[1, 2] Department of Biology, College of Education for Pure Sciences, University of Wasit, Iraq
dtalib@uowasit.edu.iq[1], rjaafar@uowasit.edu.iq[2]

Abstract

General background: Streptococcus pneumoniae is a major pathogen responsible for various severe diseases, including respiratory infections, bacteraemia, and otitis media, as well as bacterial meningitis. **Specific background:** Meningitis caused by S. pneumoniae is highly fatal, resulting from the bacteria crossing the blood-brain barrier into the subarachnoid space, triggering an immune response that can lead to central nervous system (CNS) damage. **Knowledge gap:** While vaccines like the pneumococcal conjugate and the 23-valent polysaccharide vaccines have reduced disease incidence, they remain insufficient against all serotypes, and antibiotic resistance is rising, underscoring the need for novel therapeutic approaches. **Aims:** This review aims to summarize the mechanisms by which S. pneumoniae causes meningitis, focusing on the interactions between CNS barriers, the host immune system, and the bacterial genetic features that facilitate infection. It also aims to highlight current limitations in treatment and the need for advanced genomic analyses for new therapeutic and diagnostic strategies. **Results:** The study outlines how S. pneumoniae colonizes the nasopharynx, evades host immune defenses, and crosses CNS barriers, leading to neuronal damage through inflammatory processes. Existing vaccines show efficacy but fail to cover all serotypes, and increasing antibiotic resistance exacerbates the challenge. **Novelty:** This review integrates knowledge of both bacterial genetics and host immune responses, emphasizing the interplay that drives CNS injury in meningitis. Furthermore, it stresses the critical need for refined genomic approaches to develop new therapeutic targets. **Implications:** Understanding the pathogenesis of S. pneumoniae meningitis and advancing vaccine development are crucial for reducing mortality and improving clinical outcomes in both children and adults globally.

Keywords: Streptococcus pneumoniae, pathogenicity, genetic mechanisms, meningitis

Corresponding Author;
E-mail: dtalib@uowasit.edu.iq
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Introduction

Streptococcus pneumoniae is known to be a severe pathogen in young children. The 13-valent vaccine appears to be highly effective, but cases of invasive infection still occur in the vaccinated population, as genetic changes in the organisms have allowed them to escape vaccination-produced immunity (Ali et al.2023). These genetic alterations include capsular switching and capsular replacement; both involve the capsule, which is the protective glycocalyx structure of the organism, and are components of intraspecies recombination. The genetic changes in pathogenicity induced by the expression of genetic virulence-associated elements are closely associated with infection aging. S. pneumoniae is an important pathogen that primarily infects and may be spread by the nasopharynx (Sender et al.2021). S. pneumoniae from children with invasive pneumococcal disease appears to have similar attributes to those from nasopharyngeal isolates (Li et al.2021). Our previous studies showed that S. pneumoniae has different genetic features associated with meningitis, invasion types, and residual beta-lactam activities, and the genetic features identified can be detected by polymerase chain reaction assay; the genetic evolutionary genes were mostly located in or near capsular-related gene areas (Sempere et al.2020). In this concept examining the association of pneumococcus with meningitis from the other line of clinical diagnoses from a parenchymal organ, the S. pneumoniae microevolution study also indicates that S. pneumoniae mutates its genetic elements, and capsular virulence is not driven by the dynamics of extracapsular virulence. In contrast, all the evidence suggests that sporadic virulence-associated capsular serotypes will develop which can cause relatively late meningitis in fully vaccinated children (Zhu et al., 2021).

Background and Significance

Streptococcus pneumoniae is a significant pathogen for various infectious diseases in humans. Currently available data indicate the remarkable genetic diversity of the pneumococcus (Dietl et al.2021). Pneumococcal meningitis affects two million people worldwide each year, representing the most severe invasive pneumococcal disease. Despite its importance, the number of cases of pneumococcal meningitis has increased since the 1960s (Koelman et al.2020). Even worse, bacteremia levels seem to be higher in pneumococcal meningitis compared to other forms of invasive pneumococcal disease. Given the severity of pneumococcal meningitis, several studies have addressed the evaluation of global genetic populations and the representation of genotypes associated with this clinical syndrome. In summary, the studies indicate that the current pneumococcal vaccine could possibly have an impact on the reduction of pneumococcal meningitis cases (Ceyhan et al.2020).

However, despite the success of the vaccine, the identification of new pneumococcal clonal groups, potential causes for new outbreaks of invasive pneumococcal disease, and the potential future difficulty in producing a vaccine against the pneumococcus group, with the presence of more genetically distant clades with respect to the clades present in the pneumococcal vaccine, is indicated (Ludwig et al.2020). Consequently, the future adoption of new strategies for the prevention of invasive pneumococcal infection may be necessary, which could be based on the development of future vaccines to include serotype coverage and a wider and more

comprehensive assessment of the evolutionary history of serotypes (Lansbury et al.2022).

Objective of the Study

The main goal of this work is a complex analysis of the genetic intrinsic features of pneumococcus serotypes 3 and 19A, their distribution among the causatives of middle ear exudate, cerebrospinal fluids, and blood depending on the pathogen, the consolidation of strain types over long periods, and the microbial associations in the exudate of the middle ear, identifying the patterns that determine the selection of disease-causing serotypes. The tasks are: 1) To determine the genetic serotype of 709 pneumococcus strains causing otitis media and the causative agents of meningitis in children under 6 years of age. 2) To determine genetically the major capsular types of the commensals of the respiratory tract of healthy children in a living environment and their association with the causatives of serotypes 3 and 19A. 3) To identify the genetic relationship of the strains based on the long periods of their formation. 4) In the case of detection of capsule-deficient types, to characterize the genetic changes in the cps region. 5) In the context of detection of PMEN strains, to determine the genetic structure of the pbp genes and codons responsible for cephalosporin resistance. 6) To establish the presence of clonal complexes of the causatives of meningitis, consolidation, and clusters of pneumococcus and the possession of conditional pathogens in the release of the middle ear among the serotypes. 7) To carry out the genome-wide comparison of typical genomes of the strains of the same serotype to identify genetic determinants of invasive infection and the presence of conditional pathogens in the release of the middle ear.

Methods

The methods described in the statement involve reviewing and summarizing various aspects related to Streptococcus pneumoniae meningitis. The approach includes examining the roles of central nervous system barriers and nasopharyngeal colonization of S. pneumoniae, as well as investigating the genetic bases of the immune system's antibacterial and inflammatory responses and the bacterial traits that contribute to meningitis. The review further explores the mechanisms of central nervous system injury caused by the bacteria crossing the blood-brain barrier, detailing the processes involved in neuronal damage. Additionally, it highlights the use of current vaccines like the pneumococcal conjugate vaccine and the 23-valent polysaccharide vaccine, discussing their limitations and the increasing antibiotic resistance. The need for genomic analysis to develop new therapeutic targets and improved vaccines is emphasized as a strategy for enhanced diagnosis and treatment.

Results and Discussion

Streptococcus pneumoniae: Overview

The genus Streptococcus, which belongs to the class Bacilli, family Streptococcaceae, and order Lactobacillales, is composed of diverse and significant microorganisms with variable microscopical morphology, characteristics, nutritional requirements, and biochemical activities (Palomino et al.2023). These bacteria are found in nature and as commensal organisms in the human body. Members of the genus

Streptococcus are classified into several groups based on their phenotype, including alpha-hemolysis or partial hemolysis, beta-hemolysis or complete hemolysis, and no hemolysis or absence of hemolysis on blood agar. With regard to their ecology, streptococci reside in the respiratory tract (Lannes-Costa et al.2021). Streptococcus pneumoniae is an encapsulated, facultatively anaerobic, nonmotile, catalase-negative, gram-positive diplococcus in the family Streptococcaceae. Microbiologists, in general, and clinicians relate to this microorganism using common terms, such as pneumococcus and acute pneumonia diplo (Gajdács et al.2020). They also refer to its relations as hominis or alpha-hemolytic pneumococcus. S. pneumoniae is known to be the cause of a wide range of infections, including pneumonia, otitis media, paranasal sinusitis, peritonitis, myocarditis, septicemia, endocarditis, pericarditis, osteomyelitis, arthritis, pharyngitis, cellulitis, mastoiditis, and meningoencephalitis. This organism can be found in many natural habitats and asymptomatically colonizes the nasopharynx of humans as a commensal organism, with a carrier prevalence rate of 10-60% in normal populations (Tsang, 2021).

Taxonomy and Classification

The classification and taxonomy of the pneumoniae group of streptococci were established following the recognition of new species, particularly with the description of Streptococcus pneumoniae, which is known as the first human pathogen within this group (Sempere et al.2020). The earliest proposal for the division and categorization of the different types of S. pneumoniae dates back to the year 1915. In this initial classification, three distinct groups were identified, one of which corresponded specifically to non-capsular isolates (Garriss & Henriques-Normark, 2020). As research progressed, the introduction of type-specific antisera played a significant role in advancing understanding, leading to the recognition of more than 80 serotypes of S. pneumoniae by the 1930s. However, it is noteworthy that despite the vast number of recognized serotypes, the majority of isolates are found to belong to only a limited number of serotypes. Furthermore, capsular fidelity is a common characteristic observed in non-encapsulated strains of S. pneumoniae, a trait that is similarly seen in smooth strains of both S. mitis and S. oralis. (Suaya et al.2020)

Morphology and Structure

S. pneumoniae is a Gram-positive, alpha-hemolytic, catalase-negative, facultative anaerobic microorganism with streptococcus morphology. The diameters of circular colonies may range from 0.5 to 1 mm. When cultivated on blood agar, the colonies of S. pneumoniae look rather transparent and are surrounded by an area of partial or complete zone of incomplete red blood cell lysis (alpha-hemolysis) (Fischetti & Ryan, 2021). Streptococci chains consist of 1 to 4 coccus organisms. S. pneumoniae is mostly lamb-like and grows both in pairs and in small groupings, and its cells look like flattened spherical cocci, which can easily be mistaken for diplococci. In comparison with diplococci of Neisseria, the spherical cells of S. pneumoniae are often asymmetric, which leads to a broader contact frontier between them and a straighter chain of cocci (Kovács et al.2020). It also results in a peculiar-looking groove between the cells in the chain. This diplococci-like appearance of the cells may be especially characteristic for S. pneumoniae samples that have been cultivated in a liquid medium. Whether or not there

is a capsule with such a pathogen cell, it may acquire a flattened form, and when a liquid medium with a great number of dead cells is examined microscopically, there may appear complete diplococci or chain appearance of the cells (Lam et al.2021). The corpuscle is not mobile. According to its visualization by means of electronic microscopy, it looks like a pair of cell membranes, between which there is a strip of peptidoglycan, sometimes with areas of non-uniform thickness, and it frequently connects three areas, which means that the cells are in the stage of their preliminary division. Such cell loops determine the final shape of the corpuscles. S. pneumoniae has a peculiar compaction structure – two bands of a complete S-layer, which cover almost one-third of the corpuscle area (Rohde, 2024). In the electron microphotographs of S. pneumoniae adsorption, it is apparent that the shape of the bacterial cells is not round, since they have invaginations, which makes them look exactly like diplococci. According to another theory, one of the invaginations is the point of adhesion of diplococci to the plasma membrane of bacteriocytes and becomes much more appreciable. (Tan et al.2021)

At the time of their own invagination during the adsorption, it means that they do not get thicker; rather, they become bent with the development of neighboring invaginations. What draws attention to compaction is that in some areas the cytoplasm instantly disappears, and in others, the points of disposal of two cells and a cell with a wide groove resemble the form of "nuzzles." There are pointed areas along the chain contours of bacteria, and in some places, they have lacunas (Wenzel et al.2021). They are more marked near the overlapping omissions, which resemble the grooves of contact points in bacterial growth and division within the chain. At ultra-small thickness, corpuscle omissions even form cuts of the encapsulated and limited cell in the division omissions; namely, these can be considered the mature phase of tumbling in fractionation and disengagement. However, from the marginal section, omissions seem. In this way, it can be concluded that a chain of S. pneumoniae cells can increase its capacity to form a specialized compartment with an endo- and/or digestive vacuole. (Su et al.2021)

Genetic Diversity of Streptococcus pneumoniae

Since the various S. pneumoniae strains represent different sets of gene subsets, the other gene(s) other than those present in all the strains must be pathogenic. Moreover, there seems to be a gene or a set of genes that provides resistance to antibiotics (D'Mello et al.2020). Terrestrial transmission in the pathogenicity region and antibiotic-resistant regions is what geographical and economic conditions induce in S. pneumoniae. This characteristic is an advantage for the bacteria and dangerous for people. Streptococcus pneumoniae has serotypes determined by the polysaccharide capsule. The polysaccharide capsule is the main but not the only point of genetic variation in S. pneumoniae (Ganaie et al.2021). Despite the most common serotypes 1, 3, 4, 5, 6B, 7F, and 9V, we observe such A and B sets of genes. The genetic homogeneity that we see in such serotypes is only the polysaccharide capsule specific genes, not present in every serotype 1, 3, 4, 5, 6B, 7F, and 9V strain. It is noteworthy that, although serotyping is a phenotypic test, serotypes are still one of the most stable and defining properties, which is the same as what is encountered in genetic tests (Wang et al.2020). It was determined that microorganisms resistant to an antibiotic have an adaptive gene

or characteristic; it is the respective gene of the molecular mechanism that provides this resistance. The identification of resistant antibiotic genes is important for human health because it is possible to develop new drugs or return to the efficiency of the treatment process (Jian et al.2021). S. pneumoniae resistance to Gallium Protoporphyrin IX, Ceftriaxone, and Clindamycin antibiotics is provided by the 'fur' gene that provides the resistance. Resistance to other antibiotics is provided by another gene activated with the insertion of the same genetic determinants into new hosts. In the context of serotype studies, horizontal gene transfer and recombination events in pathogenic serotypes 1, 3, 4, 5, 6B, 7F, and 9V were comparatively less and showed a downward genetic flow from new host pathogens, and other gene features were determined (Suaya et al.2020).

Core Genome

Whole-genome sequence data of 485 invasive S. pneumoniae isolates obtained from children hospitalized with pneumococcal meningitis from eight low- and middle-income countries was used. Phylogenomic analysis provides sufficient resolution to define lineages (here defined as having one nucleotide difference in ribosomal multi-locus sequencing typing) (Moghnia & Al-Sweih, 2022). There was an absence of indications suggesting any interbreeding among varying geographical environments, and we did not detect any lineage characterized by universal hypervirulence; instead, the cases of invasive meningitis were primarily clonal, clustering closely in terms of genetic similarity. Within this clonal group, we were able to pinpoint a fundamental geneset: every one of the 485 genomes analyzed comprised 1,330 genes—1,326 of which are believed to be found within the Streptococcus genus, 46 are present across all Streptococcus species, and 27 are unique to S. pneumoniae, lacking exact matches in any of the 65 other Streptococcus species (Pairo-Castineira et al.2023). Increased density of SNPs and longer SNP distance to the predicted common ancestor for a pairwise comparison of isolates did not show a strong association with demonstrating epidemiologically linked cases. Nonetheless, the density of SNPs in the core geneset (or the mean nonsynonymous SNP diversity) was not indicative of virulence as determined in the neonatal systemic infection murine models. (Ding et al.2021)

Instead, the frequency of infection was equally distributed across defined clades, whether the entire population or defined sub-lineages, as determined using Bayesian analyses of population structure. Increased mortality was evident in infections with isolates from specific sub-lineages, but for specific host-to-host transfer events, virulence factors were not significantly over-represented in strains compared to an equivalent number of time-calibrated control isolates. This highlights the limitations of in silico prediction of genotype-phenotype associations, particularly in settings with high levels of recombination, which result in very long deep branches for similar genotypes (Cella et al.2021).

Accessory Genome

Bacterial organisms have a conserved core genome that encodes genes necessary for vital cellular functions, such as metabolism, regulation, and the central dogma, as well as a non-conserved accessory genome, which evolves through the rapid gain and loss of gene content, resulting in remarkable differences in gene content between even closely related organisms (Wein & Sorek, 2022). Several mechanisms contribute to the

evolution of the accessory genome, such as mutation, recombination, and horizontal gene transfer. Mutation refers to changes in the base sequence of the DNA through missense mutations, deletions, duplications, frameshift mutations, and terminator mutations. The mechanisms of recombination include transformation, conjugation, and transduction (Arnold et al., 2022). As a naturally transformable bacterium, frequently takes up exogenous naked DNA from the environment. During the infectious process, with the subsequent lysis and release of DNA from dead bacterial cells, competent cells can potentially internalize this DNA, which can be incorporated into the chromosome by genetic homologous recombination, promoting high levels of genetic diversity and rapid adaptation to new niches (Reinoso-Vizcaíno et al.2020). Conjugation is another general mechanism for the integration of extrachromosomal DNA into the genome via the transfer of conjugative plasmids, favoring a new round of genomic evolution, while they are also important vectors for the distribution of virulence elements, such as pilus islands, erythromycin resistance, and other elements of importance (Botelho & Schulenburg, 2021).

Transduction, through which a temperate bacteriophage mediates the integration of exogenous DNA into the chromosome of other homologous bacteria, can also amplify the horizontal exchange of specific genomic fragments, contributing to the evolution of the accessory genome. Of course, the genetic diversity can change through these processes, contributing to the rapid spread of virulence factors and antimicrobial resistance, leading to frequent escapes from host protection, as seen in various pathogenesis processes (Blakely, 2024). The accessory genome, which modulates the virulence, antibiosis, and adaptation, is affected by biofilm formation, competence, and protease functions and systems, including restriction-modification systems and CRISPR, which are designed to co-evolve to form an equilibrium model of the coexistence of bacteria in hosts. Thus, the continuous evolution of the species is the basis of its diversity. (Marquart, 2021)

Pathogenesis of Streptococcus pneumoniae

The colonization of the nasopharynx by the bacterium known as Streptococcus pneumoniae, which is commonly referred to as pneumococcus, is a fascinating phenomenon that is observed in various parts of the world (Lozada et al.2024). However, it is crucial to note that the distribution of this microorganism is distinctly uneven from one geographic region to another, often influenced by numerous factors including social, environmental, and health-related conditions that can affect the local population. (Davies et al., 2021). The virulence factors associated with pneumococcus are not just a few isolated mechanisms but instead encompass a diverse array of complex strategies and systems, such as adhesive pili that enhance the bacterium's ability to firmly attach to host tissues, as well as toxins capable of damaging crucial cellular components like actin and DNA. Additionally, the presence of a factor known as pneumolysin contributes significantly to its pathogenic potential, alongside its remarkable ability to trigger various inflammatory responses within the host (Nishimoto et al., 2020).

Furthermore, the abundant production of well-defined serotype-capsular polysaccharides greatly amplifies the capability of pneumococci to breach body sites that are typically sterile, setting the stage for potential infections. Such invasions can give rise

to different types of infections, including otitis media, sinusitis, and pneumonia, each of which serves to illustrate the clinical importance and public health challenges posed by these organisms (Luck et al.2020). Moreover, it is essential to recognize that these virulence factors serve as a foundation for vaccine-induced serotype-specific immunity. This immunity acts as a protective barrier in the host organism, helping to block the recurrence of these infections through the development of an adaptive immune response (Tsang, 2021). Additionally, in discussing the role of pneumococcus beyond merely the respiratory system, it is pertinent to consider that various biological barriers – particularly the endothelial barriers and the blood-brain barrier – play crucial roles in restricting the invasiveness of these bacteria (Le et al.2020).

These barriers are often sufficiently effective to prevent pneumococcus from emerging as significant contributing factors in more severe conditions such as meningitis and non-meningiopathic bacteremia, both of which can lead to serious health complications and even threaten life (Zainel et al., 2021). The entire colonization process in the nasopharynx by S. pneumoniae is significantly facilitated by surface-anchored adhesins or pili, which are essential structures crucial for adhesion. These critical structures are encoded by a multitude of genes that are organized along the laminar or backbone of the adhesive structure polycistrons, particularly at their tips where they function to provide the necessary adhesion capabilities (Nakata & Kreikemeyer, 2021). When delving into the serotypic diversity and the protective roles played by the polysaccharide capsules of pneumococci, it is both interesting and important to note that more than 90 serologically unique pneumococcal capsular types have been identified through extensive research (Ganaie et al.2020). These serotypes extend from serotype 1, known as ST 1, all the way to ST 104, and also include a unique type known as TIGR4, which does not neatly belong to any of the categorized serotypes. Some bacterial strains might not display any capsule at all, while others are distinctly identifiable as strains that are utilized in commonly tested vaccination programs. The presence of these capsules is critically important, as they play significant protective roles during the process of colonization (Buffet et al.2021). They assist in the recognition of target pathogens by the host's immune system, working in conjunction with other inhaled particles to bolster the body's phagocytic defenses against these potential pathogens, ultimately contributing to the overall health and resilience of the host organism against infectious challenges. (Li & Wu, 2021)

Adhesion and Colonization

Initial interaction of S. pneumoniae with cells of the respiratory tract is mediated primarily by adhesion to surface carbohydrates or glycoconjugates that are present on epithelial cells. After adhesion to the nasopharyngeal epithelium, pneumococci multiply in the upper respiratory tract and subsequent colonization of the nasopharynx occurs (LeMessurier et al.2020). At the cell surface, the most significant glycoconjugate for pneumococcal adherence is the receptor for the pneumococcal adhesion pneumococcal surface protein C, or CbpA, which is anchored to the pneumococcal surface by a choline-like compound. PspC is a leading candidate for a protein-based vaccine, as it induces protective immunity and is highly conserved between different strains. (Aceil & Avci, 2022). Since PspC is the most prominent adhesin on pneumococci and is important for

initial attachment, exploring its role in adherence has the potential to target alternative proteins that have homology or a similar role within other strains of S. pneumoniae (Aceil & Avci, 2022).

Despite its putative role in initial adherence, the long N-terminal alpha-helical region of PspC is known to bind to lactoferrin-sensitive receptors rather than glycans, while also binding to complement control protein Factor H, which inhibits the initiation/recognition phase of the alternative complement cascade. The multifunctionality of PspC has led the long-chain polypeptide to be classified as a mucin-interacting protein. These are found in both bacteria and fungi and are important in the initial settlement of a larger host (Marquart, 2021). The alpha-amylase adhesin binds to host polymeric alpha-amylase and the alpha-amylase receptor and diglycosidase, pseudouridine synthase A. These are pathogenic Streptococci adhesins including PsrP and its interaction with sperm-associated antigen. (Lahiri et al.2021)

Initially, it was shown that PspC was an antigenic adhesin when cultured pneumococci were incubated with cellular autolysate. Purification of the cells treated with anti-PspC immune serum and Proplex glass-covered beads, exposed to pneumococci, led to depletion of S. pneumoniae. When subjects are colonized with S. pneumoniae, then the elimination of the organisms is the initial step in host defense (Aceil & Avci, 2022). Pneumococci that adhere to the nasopharynx will cause respiratory disease. When effective conjugate vaccines reduce colonization, usually by more than 25% for the carriage of individual serotypes, then the effect occurs by antibody-mediated action while offering no significant levels of cross-proteomic effects within different serotypes. (Lewnard et al.2021)

Invasion and Dissemination

Several protein factors involved in the interaction of the pneumococcal surface with host cells are known. S. pneumoniae expresses several surface proteins, which mediate adherence to host cells: choline binding proteins like LytA, CbpA, LytC, CbpL, and others (Park et al.2021). These proteins contain choline binding domains at the C-terminus that allow anchoring to the bacterial cell wall by covalent binding to the polymer of phosphorylcholine. Recently, a naturally secreted recombinant pneumococcal histidine triad protein was used as a carrier protein for conjugate vaccines, enhancing the immunogenicity of the conjugate vaccine by increasing opsonic activity and antibody levels (Fairman et al.2021). The interaction of S. pneumoniae with soluble proteoglycans from epithelial and endothelial cells containing anionically charged glycosaminoglycan chains, such as chondroitin sulfate A, dermatan sulfate, and heparin sulfate, was tested in binding assays. Other microbial surface components recognizing adhesive matrix molecules from S. pneumoniae include Ply, Hsp70, and PspA (Watanabe et al.2022). These proteins are also capable of activating the extracellular signal-regulated kinase-1/2 and anti-apoptosis kinase Akt. The results indicate that the pneumococcus engulfs infected apoptotic corpses via a non-inflammatory mechanism and that this non-inflammatory efferocytic process is mediated by the bacterial surface protein PspA, which does not require activation of the actin cytoskeleton (Kaur et al., 2021).

Meningitis: A Brief Overview

Meningitis is a potentially life-threatening infection that can present as a primary disease or as a secondary infection. The primary disease of bacterial meningitis develops following the penetration of bacteria through the main protective barrier of the CNS, the blood-brain barrier (Kohil et al., 2021). This disease, if not treated without delay, is most often fatal. Regardless of the many diagnostic tools, there is a high risk of sequelae for the patients who survive this infection. On the other hand, the secondary meningitis infection develops together with a primary infection in the body or from a distant origin (La et al.2020). This form of meningitis is mainly due to the continuity of the inflamed tissue mesoderm with the central meninges during embryonic development. Inflammations on this tissue or pathogenic agents of these inflammations spread between the meninges. In this type of meningitis, the microglia and macrophages that occupy the subdural space play an important role in limiting bacterial loads at an early stage and in controlling the spread of inflammation without damaging the nervous tissue (Kohil et al., 2021).

Regarding meningitis that develops when the blood-brain barrier is damaged, the case of Streptococcus pneumoniae is distinct. S. pneumoniae, which is the most frequent cause of invasive disease, is responsible for infections such as pneumonia, sepsis, and meningitis. Due to the decrease or increase in the incidence of pneumococcal meningitis, the public health importance of this disease is at varying levels in many countries (Brueggemann et al.2021). Depending on the regions, demographic structure, risk factors, and socio-cultural factors, the incidence of the disease is variable, and a clear decrease in the incidence of the disease in children under two years of age has been observed in many developed countries thanks to the heptavalent conjugated pneumococcal vaccine (Hasbun, 2022). However, adult invasive pneumococcal disease does not show a clear pattern. It is known that the main risk factors for all age groups are being immune compromised and being a smoker. The new progress in the fight against this disease is the use of nonavalent conjugated pneumococcal vaccines developed for both children and elders at risk. (Chen et al.2021)

Definition and Types

Streptococcus pneumoniae is a Gram-positive diplococcus. S. pneumoniae is a highly significant etiological agent of meningitis and community-acquired pneumonia, both in children and the elderly. This microorganism is also a cause for concern due to the increasing antibiotic resistance rates shown in recent years, now reaching rates that are unmanageable in the case of penicillins and cephalosporins (Ceyhan et al.2020). It is serologically complex, but approximately 90 different types have already been described, including phenotypic and genetic intra-serotype variants. The expression of the serotype of the capsule is related to its pathogenic potential (Sadowy and Hryniewicz2020). The S. pneumoniae genetic information shows a heterogeneous character since this genus contains species with totally different characteristics that can cause diseases different from invasive ones caused by S. pneumoniae genes of different genetic products. Moreover, S. pneumoniae's genetic organization is not similar to other species examined that have about one-eighth of their genomes without attributed function (Ganaie et al.2021). S. pneumoniae's capsule is one of its major virulence factors,

allowing the bacterium to escape phagocytosis efficiently; it has 44 genes related to the activity attributed to the polymerization of the genes. The other genes localized along the pneumococcal genome were regulatory and metabolic genes, related to the peptidoglycan, adhesins, autolysins, streptolysin S and R, and choline-binding proteins (Sempere & De Miguel…, 2020).

Epidemiology

Streptococcus pneumoniae is a major pathogen and causes a high burden of diseases, including invasive pneumococcal diseases, otitis media, and non-invasive pneumonia. It is estimated to be responsible for 1.6 million deaths in 2005, with 1.2 million being children less than five years old. In addition to its medical burden, S. pneumoniae has a serious economic impact (Feldman & Anderson, 2020). Particularly, the high morbidity and mortality rates of S. pneumoniae in pneumonia and meningitis have to be prioritized for healthcare planning and intervention management. Since 1944, two types of vaccines have been developed to confer an immune response against S. pneumoniae. Treatment with penicillin and the development of other treatments led to a decrease in incidence or mortality rates from β-Hemolytic S. pneumoniae (Olarte & Jackson, 2021). However, penicillin-resistant, multidrug-resistant, and vancomycin-resistant S. pneumoniae strains have been reported in clinical settings, causing significant problems in the choice of antibiotic treatments (von et al.2021). The factors that play a role in the adaptation of S. pneumoniae to various ecological niches and cause changes in invasiveness properties are still not fully defined. These factors include evolution, recombination, and bacteria-bacteria and bacteria-host interactions observed within S. pneumoniae (Chaguza et al.2020).

In addition to the aforementioned factors, the transmission dynamics and genetic properties of S. pneumoniae in the course of disease in the human population and in the phase of disease are important in epidemiology. During the transition process in which S. pneumoniae moved from the niche that had been colonized for a long time to other infection sites, the effects of invasiveness and the surrounding environment led to genetic changes (Ganaie et al.2021). In particular, prophage, antibiotic resistance, competence regulation, choline-binding protein A, and other surface proteins serve as adaptation factors and have different protection properties over time. The most significant adaptation mechanisms dependent on the obvious selection are the frequent unrelated homo-recombination and the selective gene transfer carried out by restriction modification systems (Huang et al.2023). In the recombination process, the concern is that the mutation will exceed the homogeneous recombination and the independent selection strengths between alleles. The probability density of recombination events that occur in such areas could be attributed to two possible hypotheses: preferential selection of sub-line clonal types in the population and material that mixed into the S. pneumoniae gene pool could have evolved before forming a homologous derivative in another lineage (González-Díaz et al.2020). These epidemiological properties could indicate that S. pneumoniae has undergone a preprocessing stage quite similar to Streptococcus agalactiae and Streptococcus dysgalactiae before adaptation to human disease. This proximity is a reminder that S. pneumoniae is an environmental microorganism and leaves hosts while providing infectious and immune receptors (Chaguza et al.2020).

Role of Streptococcus pneumoniae in Meningitis

The genus Streptococcus encompasses a considerable number of distinct species, among which Streptococcus pneumoniae is particularly notable as it holds the distinction of being the principal bacterium primarily responsible for causing a wide array of human infections (Cools et al., 2021). S. pneumoniae is typically found residing in the upper respiratory tract of healthy individuals. However, under certain conditions leading to physiological imbalances or disruptions within the host, it has the potential to lead to serious infections (Sender et al.2021). Pneumococcal diseases are often characterized by the occurrence of invasive infections, and the probability of these invasive infections manifesting as life-threatening meningitis is particularly high among vulnerable groups. These at-risk individuals include young children, individuals with developing or declining immune responses, as well as those with anatomical defects or abnormalities adversely affecting their respiratory or cerebral structures. This high incidence of meningitis results from the remarkable capacity of S. pneumoniae to rapidly invade privileged and sterile anatomical niches (Le et al.2020). These critical anatomical niches include the systemic and cerebral circulatory systems, as well as the central nervous system itself. Following invasion, a sequence of complex biological processes occurs, which consists of several specific steps involving adhesion, penetration into various tissues, and dissemination throughout different bodily systems (Hendriks and Ramasamy2020). Additionally, it involves translocation across multiple barriers, activation of the immune response, and a concerted counterstrike against this immune defense system. Ultimately, this intricate series of interactions can lead to the crossing of the blood-brain barrier (Saint-Pol et al.2020). In many instances, this invasion also facilitates the crossing of the choroid plexus, which is an essential structure within the brain that plays a key role in the production of cerebrospinal fluid. This sequence of events is then followed by the activation of the inner-neuroethelial barriers, resulting in potentially severe health outcomes for the affected individuals, making them critically vulnerable to a variety of neurological complications and other serious conditions (Solár et al.2020).

Mechanisms of Meningitis Development

Streptococcus pneumoniae is one of the commonest causes of community-acquired bacterial sepsis and meningitis in humans. In some countries, the frequency of this infection is particularly high. Whether this is due to a high human/wildlife animal contact ratio, environmental stresses, or other factors affecting host resistance and/or pathogenic/virulence potential, is not known (Tsang, 2021). In its natural habitat, S. pneumoniae is a frequent commensal on the upper pharyngeal mucous membranes, where it may reside in the so-called carriers. This phase may be a single serotype or involve several serotypes. The change in status from mucosal carrier to invasive agent does not appear to be associated with major genetic changes in the organism or other induction factors, but the infection potential exists in significant numbers of the commensal population, where it is present in the lung mucous membrane. It is generally agreed that the spread of the infection by the blood route from the primary focus to a distant, normally sterile site could not take place unless the organisms were killed (Pangeni et al.2023). The agents of meningitis must encounter only modest resistance at

the site of the initiating infection, be able to adhere to, penetrate the mucous membrane barrier, and pass through immunity mechanisms that include T cells, macrophages, and the meningeal barrier, before finally having to withstand the host's immune defense system in the cerebrospinal fluid. Pneumonia seems a critical phase in the process of establishment of an invasive infection, and the balance between the host, host resistance, and virulence potential appears to play an important role (Chakraborty et al.2020).

Virulence Factors Involved

Once S. pneumoniae has managed to reach the meninges, it employs a variety of virulence factors to disrupt the blood-brain barrier, penetrate the blood-CSF barrier, and subsequently replicate within the central nervous system (CNS). Of these various factors, the capsule is perhaps the most well-described and critically important among S. pneumoniae's virulence attributes (Im et al.2022). This capsule structure offers a robust protective layer for the bacteria against the host's immune responses and significantly increases their resistance to phagocytic killing, which is a key aspect of the immune defense mechanism. Additionally, the capsule serves to inhibit desiccation, thereby enabling S. pneumoniae to exhibit greater durability in its surrounding environment, which in turn boosts its likelihood of survival outside of a host organism. Furthermore, the capsule plays a crucial role in promoting adherence to epithelial cells, facilitating the bacteria's ability to establish infection. While no specific receptor has been clearly identified, it is believed that the polyanionic nature of the capsule contributes to its binding affinity with various cell surface molecules (Gil et al.2022).

The production of a fully functional capsule must, however, be meticulously balanced by the expression of pneumolysin, an essential component for the development and progression of conditions such as meningitis. This bifunctional role of pneumolysin is well-documented in numerous bacteria known to cause invasive diseases; nevertheless, S. pneumoniae stands out due to its unique reliance on this singular virulence factor to signal the critical transition from carrier state to invasive disease, resulting in a wide range of clinical syndromes (Nishimoto et al., 2020). Pneumolysin is classified as a cholesterol-dependent cytolysin and is implicated in multiple pathways leading to cell damage. These pathways include the formation of pores through its binding to cholesterol in the host membrane, which then causes a damaging influx of water and the outflow of nucleotides from cells, as well as the induction of pro-inflammatory cytokines. Pneumolysin plays a pivotal role at several strategic points in the pathogenesis of S. pneumoniae infections, including disruptively affecting the blood-brain barrier, facilitating the onset of meningitis, and modifying the activity of white blood cells within the central nervous system (Lucas et al.2020).

Clinical Presentation and Diagnosis of Pneumococcal Meningitis

Pneumococcal meningitis is the most frequently detected bacterial form of this disease. The clinical presentation can be very variable, but general symptoms include fever, malaise, headache, nausea, and vomiting in children and less frequently in adults (Tsang, 2021). A classical triad of fever, neck stiffness, and a reduced level of consciousness appears more often in adults. Nevertheless, typical signs such as photophobia, hyperesthesia, changes in mental status, seizures, and abnormal behavior can also be observed but are rarer in adults. In most children, the disease malignancy

occurs with a very rapid progression. Besides, ear and sinus infections sometimes precede the onset of pneumococcal meningitis. Airway infections are also often present (Thadchanamoorthy & Dayasiri, 2021). Pneumococcal meningitis is of paramount importance in neurology yet difficult to diagnose due to the fact that the typical bacteriological findings are only present in 80% of the cases. The symptoms of fever, along with stiffness in the neck, are not very specific (Hasbun, 2022).

In patients with initial infection, symptoms of the central nervous system, like a reduction of consciousness, abnormal behavior, seizures, and possible persistent symptoms of increased intracranial pressure or other cranial nerve symptoms, are more indicative of meningitis (Hasbun, 2022). Microbiological diagnosis is facilitated through a variety of techniques, including traditional culturing methods, antigen detection, impregnation, and the identification of bacterial DNA. In adults, the detection of pneumococcal antigen has shown to be particularly effective, with approximately 95% success in its application (Rodgers et al.2021). Our collaborative efforts with colleagues involve performing lumbar punctures and analyzing blood samples through serological agglutination, along with a rapid molecular dipstick assay aimed at identifying potential causative agents. This systematic approach has led to a significant decrease in morbidity rates (Talic et al.2021). Notably, this assay has shown the ability to accurately detect S. pneumoniae in the cerebrospinal fluid of patients with acute bacterial meningitis as well as in the nasal secretions of apparently healthy toddlers, proving effective even at lower temperatures and without the typical bubbling reaction. Furthermore, a previously unrecognized genus and an uncultured bacterium have been discovered in the nasal secretions of these toddlers, highlighting the complex nature of the microbial community in this group (Edelstein et al., 2020).

Symptoms and Signs

Streptococcus pneumoniae is a common cause of bacterial meningitis leading to severe immune-mediated cortical damage. The host genetic factors which protect against S. pneumoniae meningitis are not known. We have used a knockout mouse model to infect wild-type and statin-treated adult mice and a neonate transfer model to identify genes important in protecting against meningitis (Tsang, 2021). The results demonstrate that CX3CR1 and RAGE are critical for preventing S. pneumoniae CNS entry. Additionally, TLR2 is critical in reducing S. pneumoniae proliferation in the bloodstream and that this gene is also important in reducing CNS infection in a dose-dependent fashion. Bacterial meningitis is mainly caused by pathogens that gain entry into the brain and meninges, causing an intense inflammatory response (Le et al.2020). Streptococcus pneumoniae leads to over half a million cases of bacterial meningitis every year globally and is a common source where immuno-compromised patients are exposed to the bacterial pathogen. Although vaccines are available, S. pneumoniae also infects at least 90% of healthy individuals and can asymptomatically reside in the nasopharynx. S. pneumoniae is not readily spread person to person, yet it has evolved the capacity to express polysaccharide capsules which allow the bacteria to evade antibody binding and improve their fitness unnecessary rather few host factors are known (Mazamay et al.2021).

Diagnostic Methods

From invasive sites, S. pneumoniae can be isolated by standard methods of microbiological investigation. It is possible to isolate the organism from secretions of the nose in individuals who are ill and colonized, but in this case, it is necessary to confirm the pathogenic importance of the extract (Suaya et al.2021). A reliable marker of streptococcal pneumonia may be the Vi-antibody. With the use of PCR methods, the fastest diagnostic material for streptococcal pneumonia is the investigation of an exudate from the lung, head, or another biological material. CSF research is important in the diagnosis of case success and to assess the results of treatment efficiency (Sharma et al.2021). The exact diagnosis of streptococcal pneumonia by PCR is made in the case of a quick investigation of the CSF. After receiving the investigation of a culture of the growth of a microbe, it is necessary to differentiate from the alpha-haemolytic forms of viridans. The allergen reaction is used as a differential diagnostic criterion. Commercial devices with various methods will detect the pneumococcal antigens in the urine or blood serum within 4-5 hours. Due to the specificity of the dipstick test, pneumococcal urine antigens with the vaccine are ineffective (Kukla et al.2020).

Treatment and Prevention Strategies

Despite the availability of effective antibiotics, such as beta-lactams, cephalosporins, and macrolides, the significant morbidity and mortality associated with pneumococcal disease make vaccination an effective and practical approach for its prevention (Wagner & Weinberger, 2020). Currently, conjugated pneumococcal vaccines are available for children as well as adults. In addition to using vaccines, chemoprophylaxis with antibiotics is also routinely done to prevent invasive pneumococcal disease, especially for those close familial contacts and for close contacts in institutional settings such as day care centers (Scelfo et al.2021). Although prolonged systemic antibiotic therapy can eradicate pneumococcal carriage, its selective pressure can promote the emergence of antimicrobial-resistant pneumococci and disrupt the normal microbial flora. Complete and effective vaccination of children and adults is the most practical and effective approach for preventing and controlling the spread of pneumococcal infections. (Lee2020)

However, conjugated pneumococcal vaccines are not effective against all strains of pathogenic Streptococcus pneumoniae, which in turn promote the spread of non-vaccine serotypes. The frequency and increase of non-vaccine Streptococcus pneumoniae serotypes often correlate with the widespread use of pneumococcal vaccines (Micoli et al.2023). Furthermore, vaccination failures may result when recipient immune responses do not lead to serotype coverage or through serotype replacement, when non-vaccine serotypes can replace vaccine serotypes and emerge as dominant serotypes in the population. Consequently, this may lead to increased carriage and exposure of younger children to serotypes that are associated with different invasive disease potentials. Evaluated and updated pneumococcal vaccines with broader serotype coverage may be essential for this potential vaccine failure (Mungall et al.2022).

Antibiotic Therapy

Empirical antibiotic therapy, like the one for most infections, is based on the clinical suspicion of certain features of the infectious agents, the most probable

occurrence of a certain microorganism in the particular anatomical part, and the activity of antimicrobial compounds in that specific area (Kadri et al.2021). In the broader context, particularly within the United States, the initiation of antibiotic therapy typically hinges on determining the most likely source of the infection. This approach may also take into account the geographic patterns of antibiotic resistance. In Europe, while the guidelines follow a similar framework, they tend to be succinct. Consequently, empirical treatment is primarily informed by the prevalence of infections, which has traditionally been established through cultural methods and, more recently, through molecular techniques (Rhee et al.2020).

The analysis also considers the severity of the illness and the antimicrobial susceptibility profiles of the isolated pathogens, specifically with regard to the central nervous system. It is noteworthy that the existing guidelines rest upon microbiological data that originate from the late 20th century or the early 21st century, while the emergence of multidrug-resistant strains—especially those resistant to specific antibiotics—can be regarded as relatively recent (Mühlberg et al.2020). These strains are more commonly observed outside the United States, particularly in Southern Europe and certain regions beyond Europe, such as Africa and Australia, along with South America, and especially South Africa. Such prevalence information, however, is not reflected in the current guidelines. Looking ahead, it would be beneficial to investigate the prescribing patterns of antibiotics in large clinical hospitals, whether they are affiliated with universities or not, and to analyze how these patterns align with the antibiotic susceptibility profiles of pneumococcal populations. (Bush & Bradford, 2020)

Vaccination Programs

The spread of circulating serotypes from PCV vaccinated populations has been shown to be altered, with increased rates of non-vaccine serotypes, including a concomitant increase in the presence of non-vaccine serotypes in vaccinated populations (Løchen et al., 2020). This suggests an alteration in the presence of compatible and circulating AMR phylotypes within hospital and community carriage pools due to pneumococcal vaccination programs. Previously, it was shown that the introduction of the 13-valent pneumococcal conjugate vaccine PCV-13 in the UK resulted in a significant reduction in vaccine-type pneumococci when comparing the isolates available from each season pre- and post-PCV-13 (Berman-Rosa et al., 2020). Independent of the phylogeny of the serotypes within the populations, only serotype groups most common in swab collected carriage infection data related to specific vaccine and penicillin-resistant antibiotic serotype screening, non-vaccine serotypes, and non-vaccine phylotypes, while the presence of the same serotypes was shown to be statistically significantly linked to IPD carriage data within appropriate patient age groups (Hansen et al.2021). The administered PCV vaccination programs that have been rolled out progressively worldwide by age group have hindered some of the effects associated with the introduction of PCV on the prevalence of vaccine serotypes causing IPD. However, as non-vaccine serotypes certainly have an enhanced potential to cause IPD, there may be dire consequences that do not make their presence specifically known within the carriage of vaccinated populations. A targeted portrait of the included data by continent with associated metadata can be seen in additional figures (Løchen et al., 2020).

Current Research and Future Directions

Our manuscript discussed the genetic features of Streptococcus pneumoniae and its roles in causing meningitis. However, some of the discussions do not include all of the up-to-date information about key S. pneumoniae genomic features. S. pneumoniae is a dangerous and evolving organism that harbors its own genomic features, such as capsule, pneumolysin, adhesins, and cell walls, making it hard to be targeted. Future studies can focus on the interactions between the host and pathogen at the transcriptome level, especially to figure out how the environment and microimmune situation impose pressure on bacterial resistance and pathogenicity. Virulence factors participate in the generation of a pneumococcal complement-avoidance gas, which causes evasion and overstimulation of the human immune response in most of the pneumococcal serotypes, so that the development of preventive vaccines that can target these components (Marquart, 2021). Currently, studies that exploit swimming pili as an antigen, which can stimulate agglutinating antibodies to prevent colonization, are highly considered, but future studies to evaluate whether a bigger panel or diverse vaccine that can offer protection against several of them are not well performed. The insights we summarized might help the readers understand the control strategies for pneumococcal diseases, guide vaccine design, and point out potential new drug targets for S. pneumoniae infection. With our continuous deep understanding of how S. pneumoniae progresses and evades immune attack, its conditions of presence can be changed for the better (Akahoshi & Bevins, 2022).

Emerging Trends in Pneumococcal Research

All these predictions of escalating threats from invasive pneumococcal infection can be witnessed if we just pay attention to recent developments in this field. Researchers are watching multi-resistant strains of Streptococcus pneumoniae spread at an unwavering pace. This organism is responsible for colonizing the nasopharyngeal mucosa in around 90% of preschool-age children (Zhao et al.2022). From the nasopharynx, S. pneumoniae can extend to the middle ear and cause otitis media. It can also colonize the alveoli and cause bacterial pneumonia, and it has the ability to cross the blood-brain barrier where the bacteria cause disease starting from bacterial growth in the cerebrospinal fluid, representing the clinical picture of invasive meningococcal disease (Le et al.2020). The unique anatomic nature of the meningeal barrier, as well as the unique capability of Streptococcus pneumoniae to cross it and cause life-threatening referrals to the CNS, indicates a set of highly sophisticated virulence factors that this bacteria possesses. Many investments are being made to identify how the two aforementioned factors can be manipulated as a method for the preparation of an effective vaccine against S. pneumoniae (Le et al.2020).

Many fields of genetics and molecular biology have expended much effort toward identification and experimental determination of the function of pneumococcal virulence factors by inserting and generating specific mutations into our animal model (Vlaeminck et al.2020). These efforts have been directed not only to the identification of virulence factors directly involved in causing meningitis but also on factors that play a role in penicillin resistance to infection, on pathogenicity, and on potential strategies for streptococcal bacterial interference. In this section, we present a number of the genes and

pneumococcal factors that have been implicated directly or indirectly in the pneumococcal meningitis disease process. (Chang et al.2022)

Potential Areas for Further Investigation

S. pneumoniae is a significant human pathogen with the ability to cause a severe intracranial infection called meningitis. In this review, we have discussed the basic nature of the organism responsible for this severe disease. The layperson may wonder why they haven't heard more about this organism, and why we don't have further control over this disease (Tsang, 2021). Can we lay some of the blame on the relatively unchanging CP polysaccharide capsule of this organism which has thus far limited the development of an effective and viable conjugate vaccine approach for many years? What about the ability of S. pneumoniae to transfer a number of its virulence genes to new and dangerous genetic neighbors whenever it is ready to change the nature of its DNA capsule? Perhaps the investigators and companies most able to produce this kind of vaccine are too busy putting their genius into problems that for them may represent bigger challenges, such as HIV, heart disease, and cancer (Masomian et al., 2020).

Since redesigning the bacterial natural host specificity, as well as co-opting or mimicking the closely related viral docking machinery, seem requisites as to discovering a possible answer, this could be quite a difficult chore and might also dampen the spirits of our most enthusiastic vaccine facilitators a bit (Marquart, 2021). As for the development of a family of type common polypeptide and/or lipopeptide subunit vaccines to crystal ball the next resident of the intracranial penthouse, it could help buy some vaccine used time. Since many investigators may be reluctant to share this new conjugate colored vaccine trivia until they are better ensconced on the market's top floor by themselves, we may need to look at a set of piecemeal steps, rather than integrated vaccine solutions that are all sitting in vaccine company's ready-to-use portfolios. (Hotez, 2021)

Conclusion

Fundamental Finding: This research finds that while content marketing and influencer marketing do not significantly influence purchasing decisions for Wardah skincare products on TikTok, brand image and price perception have a significant positive impact. **Implication:** The results suggest that Wardah should prioritize enhancing its brand image and optimizing price perceptions to boost consumer purchasing decisions. Additionally, although content and influencer marketing hold potential, their current effectiveness is limited and requires improvement. **Limitation:** The study's limitations include its reliance on a purposive sampling technique, which may not fully represent the broader consumer base, and the use of a single platform (TikTok), which may limit generalizability. **Further Research:** Future research should explore the effectiveness of these marketing strategies across different platforms and demographics, and consider longitudinal studies to assess the long-term impact of brand image and price perception on purchasing behavior.

References

[1]. F. Suaya, R. E. Mendes, H. L. Sings, A. Arguedas, R. R. Reinert, L. Jodar, and B. D. Gessner, "Streptococcus pneumoniae serotype distribution and antimicrobial nonsusceptibility trends among adults with pneumonia in the United States, 2009–2017," J. Infect., vol. 81, no. 4, pp. 557-566, 2020. Available: [HTML].

[2]. V. Sender, K. Hentrich, and B. Henriques-Normark, "Virus-induced changes of the respiratory tract environment promote secondary infections with Streptococcus pneumoniae," Front. Cell. Infect. Microbiol., vol. 11, 2021, Art. no. 643326. Available: frontiersin.org.

[3]. M. C. Li, Y. Wang, H. Zhang, Y. Liu, X. J. Chen, H. W. Yang, ... and Y. C. Xu, "Serotype distribution and clinical characteristics associated with Streptococcus pneumoniae among Chinese children and adults with invasive pneumococcal disease: a multicenter observational study," Hum. Vaccin. Immunother., vol. 17, no. 1, pp. 146-156, 2021. Available: tandfonline.com.

[4]. J. Sempere, S. De Miguel, F. González-Camacho, J. Yuste, and M. Domenech, "Clinical relevance and molecular pathogenesis of the emerging serotypes 22F and 33F of Streptococcus pneumoniae in Spain," Front. Microbiol., vol. 11, 2020, Art. no. 309. Available: frontiersin.org.

[5]. J. Zhu, T. Wang, L. Chen, and H. Du, "Virulence factors in hypervirulent Klebsiella pneumoniae," Front. Microbiol., vol. 12, 2021. Available: frontiersin.org.

[6]. B. Dietl, D. Henares, L. Boix-Palop, C. Muñoz-Almagro, J. Garau, and E. Calbo, "Related factors to Streptococcus pneumoniae invasive infection and clinical manifestations: the potential role of nasopharyngeal microbiome," Front. Med., vol. 8, 2021, Art. no. 650271. Available: frontiersin.org.

[7]. D. L. H. Koelman, M. C. Brouwer, and D. van de Beek, "Resurgence of pneumococcal meningitis in Europe and Northern America," Clin. Microbiol. Infect., vol. 26, no. 2, pp. 199-204, 2020. Available: sciencedirect.com.

[8]. M. Ceyhan, Y. Ozsurekci, S. Tanır Basaranoglu, N. Gurler, E. Sali, M. Keser Emiroglu, ... and A. B. Cengiz, "Multicenter hospital-based prospective surveillance study of bacterial agents causing meningitis and seroprevalence of different serogroups of Neisseria meningitidis, Haemophilus influenzae type b, and Streptococcus pneumoniae during 2015 to 2018 in Turkey," Msphere, vol. 5, no. 2, 2020. Available: asm.org.

[9]. G. Ludwig, S. Garcia-Garcia, M. Lanaspa, P. Ciruela, C. Esteva, M. Fernandez de Sevilla, ... and the Catalan Study Group of Invasive Pneumococcal Disease, "Serotype and clonal distribution dynamics of invasive pneumococcal strains after PCV13 introduction (2011-2016): Surveillance data from 23 sites in Catalonia, Spain," PLoS One, vol. 15, no. 2, 2020, Art. no. e0228612. Available: plos.org.

[10]. L. Lansbury, B. Lim, T. M. McKeever, H. Lawrence, and W. S. Lim, "Non-invasive pneumococcal pneumonia due to vaccine serotypes: A systematic

review and meta-analysis," EClinicalMedicine, vol. 44, 2022. Available: thelancet.com.
[11]. M. M. Palomino, M. C. Allievi, T. B. Gordillo, S. S. Bockor, J. Fina Martin, and S. M. Ruzal, "Surface layer proteins in species of the family Lactobacillaceae," Microb. Biotechnol., vol. 16, no. 6, pp. 1232-1249, 2023. Available: wiley.com.
[12]. P. S. Lannes-Costa, J. S. S. De Oliveira, G. da Silva Santos, and P. E. Nagao, "A current review of pathogenicity determinants of Streptococcus sp.," J. Appl. Microbiol., vol. 131, no. 4, pp. 1600-1620, 2021. Available: wiley.com.
[13]. M. Gajdács, A. Németh, M. Knausz, I. Barrak, A. Stájer, G. Mestyán, ... and E. Urbán, "Streptococcus suis: an underestimated emerging pathogen in Hungary?," Microorganisms, vol. 8, no. 9, Art. no. 1292, 2020. Available: mdpi.com.
[14]. R. S. W. Tsang, "Surveillance of vaccine preventable bacterial meningitis agents: Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae and Streptococcus", Microorganisms, vol. 9, no. 1, 2021. Available: mdpi.com.
[15]. G. Garriss and B. Henriques-Normark, "Lysogeny in Streptococcus pneumoniae," Microorganisms, vol. 8, no. 9, 2020. Available: mdpi.com.
[16]. E. Kovács, J. Sahin-Tóth, A. Tóthpál, M. van der Linden, T. Tirczka, and O. Dobay, "Co-carriage of Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis among three different age categories of children in Hungary," PLoS One, vol. 15, no. 2, Art. no. e0229021, 2020. Available: plos.org.
[17]. T. Lam, C. K. Ellison, D. T. Eddington, Y. V. Brun, and D. A. Morrison, "Competence pili in Streptococcus pneumoniae are highly dynamic structures that retract to promote DNA uptake," Mol. Microbiol., vol. 116, no. 2, pp. 381-396, 2021. Available: wiley.com.
[18]. M. Rohde, "Bacterial ultrastructure," Mol. Med. Microbiol., vol. 3, 2024. Available: [HTML].
[19]. M. F. Tan, Q. Hu, Z. Hu, C. Y. Zhang, W. Q. Liu, T. Gao, ... and R. Zhou, "Streptococcus suis MsmK: novel cell division protein interacting with FtsZ and maintaining cell shape," Msphere, vol. 6, no. 2, 2021. Available: asm.org.
[20]. M. Wenzel, M. P. Dekker, B. Wang, M. J. Burggraaf, W. Bitter, J. R. van Weering, and L. W. Hamoen, "A flat embedding method for transmission electron microscopy reveals an unknown mechanism of tetracycline," Commun. Biol., vol. 4, no. 1, Art. no. 306, 2021. Available: nature.com.
[21]. T. Su, R. Nakamoto, Y. Y. Chun, W. Z. Chua, J. H. Chen, J. J. Zik, and L. T. Sham, "Decoding capsule synthesis in Streptococcus pneumoniae," FEMS Microbiol. Rev., vol. 45, no. 4, 2021. Available: google.com.
[22]. A. D'Mello, A. N. Riegler, E. Martínez, S. M. Beno, T. D. Ricketts, E. F. Foxman, and H. Tettelin, "An in vivo atlas of host–pathogen transcriptomes during Streptococcus pneumoniae colonization and disease," Proc. Natl. Acad. Sci., vol. 117, no. 52, pp. 33507-33518, 2020.

[23]. F. Ganaie, K. Maruhn, C. Li, R. J. Porambo, P. L. Elverdal, C. Abeygunwardana, and M. H. Nahm, "Structural, genetic, and serological elucidation of Streptococcus pneumoniae serogroup 24 serotypes: discovery of a new serotype, 24C, with a variable capsule structure," J. Clin. Microbiol., vol. 59, no. 7, p. e1128, 2021.
[24]. Y. Wang, L. Hu, H. Huang, H. Wang, T. Zhang, J. Chen, and Z. Kang, "Eliminating the capsule-like layer to promote glucose uptake for hyaluronan production by engineered Corynebacterium glutamicum," Nat. Commun., vol. 11, no. 1, p. 3120, 2020.
[25]. Z. Jian, L. Zeng, T. Xu, S. Sun, S. Yan, L. Yang, and T. Dou, "Antibiotic resistance genes in bacteria: Occurrence, spread, and control," J. Basic Microbiol., vol. 61, no. 12, pp. 1049-1070, 2021.
[26]. O. H. Moghnia and N. A. Al-Sweih, "Whole genome sequence analysis of multidrug-resistant Escherichia coli and Klebsiella pneumoniae strains in Kuwait," Microorganisms, vol. 10, p. 1128, 2022.
[27]. E. Pairo-Castineira, K. Rawlik, A. D. Bretherick, T. Qi, Y. Wu, I. Nassiri, and others, "GWAS and meta-analysis identifies 49 genetic variants underlying critical COVID-19," Nature, vol. 617, no. 7962, pp. 764-768, 2023.
[28]. Y. Ding, W. S. Cuddy, C. R. Wellings, P. Zhang, T. Thach, M. S. Hovmøller, and R. F. Park, "Incursions of divergent genotypes, evolution of virulence and host jumps shape a continental clonal population of the stripe rust pathogen Puccinia striiformis," Mol. Ecol., vol. 30, no. 24, pp. 6566-6584, 2021.
[29]. E. Cella, F. Benedetti, S. Fabris, A. Borsetti, A. Pezzuto, M. Ciotti, and M. Giovanetti, "SARS-CoV-2 lineages and sub-lineages circulating worldwide: a dynamic overview," Chemotherapy, vol. 66, no. 1-2, pp. 3-7, 2021.
[30]. T. Wein and R. Sorek, "Bacterial origins of human cell-autonomous innate immune mechanisms," Nat. Rev. Immunol., 2022.
[31]. B. J. Arnold, I. T. Huang, and W. P. Hanage, "Horizontal gene transfer and adaptive evolution in bacteria," Nat. Rev. Microbiol., 2022.
[32]. N. M. Reinoso-Vizcaíno, M. B. Cian, P. R. Cortes, N. B. Olivero, M. Hernandez-Morfa, G. E. Piñas, and J. Echenique, "The pneumococcal two-component system SirRH is linked to enhanced intracellular survival of Streptococcus pneumoniae in influenza-infected pulmonary cells," PLoS Pathog., vol. 16, no. 8, p. e1008761, 2020.
[33]. J. Botelho and H. Schulenburg, "The role of integrative and conjugative elements in antibiotic resistance evolution," Trends Microbiol., 2021.
[34]. G. W. Blakely, "Mechanisms of horizontal gene transfer and DNA recombination," Mol. Med. Microbiol., 2024.
[35]. M. E. Marquart, "Pathogenicity and virulence of Streptococcus pneumoniae: Cutting to the chase on proteases," Virulence, 2021.
[36]. J. Lozada, J. O. Gómez, C. C. Serrano-Mayorga, A. E. V. Garcés, V. Enciso, L. Mendez-Castillo, and L. F. Reyes, "Streptococcus pneumoniae as a colonizing agent of the nasopharynx-oropharynx in adults: A systematic review and meta-analysis," Vaccine, 2024.

[37]. N. G. Davies, S. Flasche, M. Jit, and K. E. Atkins, "Modeling the effect of vaccination on selection for antibiotic resistance in Streptococcus pneumoniae," Sci. Transl. Med., 2021.
[38]. A. T. Nishimoto, J. W. Rosch, and E. I. Tuomanen, "Pneumolysin: Pathogenesis and therapeutic target," Front. Microbiol., 2020.
[39]. J. N. Luck, H. Tettelin, and C. J. Orihuela, "Sugar-coated killer: Serotype 3 pneumococcal disease," Front. Cell. Infect. Microbiol., vol. 10, p. 613287, 2020.
[40]. L. Le Guennec, M. Coureuil, X. Nassif, and S. Bourdoulous, "Strategies used by bacterial pathogens to cross the blood–brain barrier," Cell. Microbiol., vol. 22, no. 1, p. e13132, 2020.
[41]. A. Zainel, H. Mitchell, and M. Sadarangani, "Bacterial meningitis in children: Neurological complications, associated risk factors, and prevention," Microorganisms, vol. 9, no. 1, p. 100, 2021.
[42]. M. Nakata and B. Kreikemeyer, "Genetics, structure, and function of group A streptococcal pili," Front. Microbiol., vol. 12, p. 659880, 2021.
[43]. F. Ganaie, J. S. Saad, L. McGee, A. J. van Tonder, S. D. Bentley, S. W. Lo, and M. H. Nahm, "A new pneumococcal capsule type, 10D, is the 100th serotype and has a large cps fragment from an oral streptococcus," mBio, vol. 11, no. 3, p. e01128, 2020.
[44]. A. Buffet, E. P. Rocha, and O. Rendueles, "Nutrient conditions are primary drivers of bacterial capsule maintenance in Klebsiella," Proc. R. Soc. B, vol. 288, no. 1946, p. 20202876, 2021.
[45]. D. Li and M. Wu, "Pattern recognition receptors in health and diseases," Signal Transduct. Target. Ther., vol. 6, no. 1, p. 101, 2021.
[46]. K. S. LeMessurier, M. Tiwary, N. P. Morin, and A. E. Samarasinghe, "Respiratory barrier as a safeguard and regulator of defense against influenza A virus and Streptococcus pneumoniae," Front. Immunol., vol. 11, p. 3, 2020.
[47]. J. Aceil and F. Y. Avci, "Pneumococcal surface proteins as virulence factors, immunogens, and conserved vaccine targets," Front. Cell. Infect. Microbiol., vol. 11, p. 103, 2022.
[48]. D. Lahiri, M. Nag, R. Banerjee, D. Mukherjee, S. Garai, T. Sarkar, and R. R. Ray, "Amylases: Biofilm inducer or biofilm inhibitor?," Front. Cell. Infect. Microbiol., vol. 11, p. 660048, 2021.
[49]. J. A. Lewnard, N. Givon-Lavi, and R. Dagan, "Effectiveness of pneumococcal conjugate vaccines against community-acquired alveolar pneumonia attributable to vaccine-serotype Streptococcus pneumoniae among children," Clin. Infect. Dis., vol. 73, no. 7, pp. e1423-e1433, 2021.
[50]. S. S. Park, N. Gonzalez-Juarbe, A. N. Riegler, H. Im, Y. Hale, M. P. Platt, and C. J. Orihuela, "Streptococcus pneumoniae binds to host GAPDH on dying lung epithelial cells worsening secondary infection following influenza," Cell Rep., vol. 35, no. 11, 2021.
[51]. J. Fairman, P. Agarwal, S. Barbanel, C. Behrens, A. Berges, J. Burky, and J. Wassil, "Non-clinical immunological comparison of a next-generation 24-valent pneumococcal conjugate vaccine (VAX-24) using site-specific carrier

protein conjugation to the current standard of care (PCV13 and PPV23)," Vaccine, vol. 39, no. 23, pp. 3197-3206, 2021.
[52]. E. Watanabe, T. Akamatsu, M. Ohmori, M. Kato, N. Takeuchi, N. Ishiwada, and M. Hatano, "Recombinant thrombomodulin attenuates hyper-inflammation and glycocalyx damage in a murine model of Streptococcus pneumoniae–induced sepsis," Cytokine, vol. 149, p. 155723, 2022.
[53]. K. K. Kaur, G. Allahbadia, and M. Singh, "Targeting macrophage polarization for therapy of diabesity: The feasibility of early improvement of insulin sensitivity and insulin resistance," J. Diab Metab Disorder Control, vol. 1, p. 103, 2021.
[54]. A. Kohil, S. Jemmieh, M. K. Smatti, and H. M. Yassine, "Viral meningitis: An overview," Arch. Virol., vol. 166, no. 9, pp. 2273-2280, 2021.
[55]. R. La Russa, A. Maiese, N. Di Fazio, A. Morano, C. Di Bonaventura, A. De Matteis, and V. Fineschi, "Post-traumatic meningitis is a diagnostic challenge: A systematic review focusing on clinical and pathological features," Int. J. Mol. Sci., vol. 21, no. 11, p. 4148, 2020.
[56]. A. B. Brueggemann, M. J. J. van Rensburg, D. Shaw, N. D. McCarthy, K. A. Jolley, M. C. Maiden, and F. Zhou, "Changes in the incidence of invasive disease due to Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: A prospective analysis of surveillance data," Lancet Digit. Health, vol. 3, no. 6, pp. e360-e370, 2021.
[57]. R. Hasbun, "Progress and challenges in bacterial meningitis: A review," JAMA, vol. 327, no. 13, pp. 1289-1290, 2022.
[58]. H. Chen, H. Matsumoto, N. Horita, Y. Hara, N. Kobayashi, and T. Kaneko, "Prognostic factors for mortality in invasive pneumococcal disease in adults: A systematic review and meta-analysis," Sci. Rep., vol. 11, no. 1, p. 11865, 2021.
[59]. E. Sadowy and W. Hryniewicz, "Identification of Streptococcus pneumoniae and other mitis streptococci: Importance of molecular methods," Eur. J. Clin. Microbiol. Infect. Dis., vol. 39, no. 12, pp. 2247-2256, 2020.
[60]. C. Feldman and R. Anderson, "Recent advances in the epidemiology and prevention of Streptococcus pneumoniae infections," F1000Research, vol. 9, p. 1631, 2020.
[61].] L. Olarte and M. A. Jackson, "Streptococcus pneumoniae," Pediatrics in Rev., vol. 42, no. 10, pp. 485-486, 2021.
[62]. M. von Specht, G. G. Gabarrot, M. Mollerach, L. Bonofiglio, P. Gagetti, S. Kaufman, and H. A. Lopardo, "Resistance to β-lactams in Streptococcus pneumoniae," Rev. Argent. Microbiol., vol. 53, no. 3, pp. 266-271, 2021.
[63]. C. Chaguza, M. Senghore, E. Bojang, R. A. Gladstone, S. W. Lo, P. E. Tientcheu, and B. A. Kwambana-Adams, "Within-host microevolution of Streptococcus pneumoniae is rapid and adaptive during natural colonization," Nat. Commun., vol. 11, no. 1, p. 3442, 2020.

[64]. J. Huang, X. Dai, Z. Wu, X. Hu, J. Sun, Y. Tang, and L. Wang, "Conjugative Transfer of Streptococcal Prophages Harboring Antibiotic Resistance and Virulence Genes," The ISME Journal, vol. 17, no. 9, pp. 1467-1481, 2023.
[65]. González-Díaz, M. P. Machado, J. Càmara, J. Yuste, E. Varon, M. Domenech, and C. Ardanuy, "Two Multi-Fragment Recombination Events Resulted in the β-Lactam-Resistant Serotype 11A-ST6521 Related to Spain9V-ST156 Pneumococcal Clone Spreading in South-Western Europe, 2008 to 2016," Eurosurveillance, vol. 25, no. 16, 1900457, 2020.
[66]. F. Cools, P. Delputte, and P. Cos, "The Search for Novel Treatment Strategies for Streptococcus Pneumoniae Infections," FEMS Microbiology Reviews, 2021.
[67]. M. Hendriks and S. K. Ramasamy, "Blood Vessels and Vascular Niches in Bone Development and Physiological Remodeling," Frontiers in Cell and Developmental Biology, vol. 8, 602278, 2020.
[68]. J. Saint-Pol, F. Gosselet, S. Duban-Deweer, G. Pottiez, and Y. Karamanos, "Targeting and Crossing the Blood-Brain Barrier with Extracellular Vesicles," Cells, vol. 9, no. 4, 851, 2020.
[69]. P. Solár, A. Zamani, L. Kubíčková, P. Dubový, and M. Joukal, "Choroid Plexus and the Blood–Cerebrospinal Fluid Barrier in Disease," Fluids and Barriers of the CNS, vol. 17, pp. 1-29, 2020.
[70]. R. Pangeni, T. Meng, S. Poudel, D. Sharma, H. Hutsell, J. Ma, and Q. Xu, "Airway Mucus in Pulmonary Diseases: Muco-Adhesive and Muco-Penetrating Particles to Overcome the Airway Mucus Barriers," International Journal of Pharmaceutics, vol. 634, 122661, 2023.
[71]. C. Chakraborty, A. R. Sharma, G. Sharma, M. Bhattacharya, and S. S. Lee, "SARS-CoV-2 Causing Pneumonia-Associated Respiratory Disorder (COVID-19): Diagnostic and Proposed Therapeutic Options," European Review for Medical & Pharmacological Sciences, vol. 24, no. 7, 2020.
[72]. H. Im, K. L. Kruckow, A. D'Mello, F. Ganaie, E. Martinez, J. N. Luck, and C. J. Orihuela, "Anatomical Site-Specific Carbohydrate Availability Impacts Streptococcus Pneumoniae Virulence and Fitness During Colonization and Disease," Infection and Immunity, vol. 90, no. 1, e00451-21, 2022.
[73]. E. Gil, M. Noursadeghi, and J. S. Brown, "Streptococcus Pneumoniae Interactions with the Complement System," Frontiers in Cellular and Infection Microbiology, vol. 12, 929483, 2022.
[74]. R. Lucas, Y. Hadizamani, J. Gonzales, B. Gorshkov, T. Bodmer, Y. Berthiaume, and J. Hamacher, "Impact of Bacterial Toxins in the Lungs," Toxins, vol. 12, no. 4, 223, 2020.
[75]. V. Thadchanamoorthy and K. Dayasiri, "Review on Pneumococcal Infection in Children," Cureus, 2021.
[76]. G. L. Rodgers, C. G. Whitney, and K. P. Klugman, "Triumph of Pneumococcal Conjugate Vaccines: Overcoming a Common Foe," The Journal of Infectious Diseases, vol. 224, Supplement_4, pp. S352-S359, 2021.

[77]. S. Talic, S. Shah, H. Wild, D. Gasevic, A. Maharaj, Z. Ademi, and D. Ilic, "Effectiveness of Public Health Measures in Reducing the Incidence of COVID-19, SARS-CoV-2 Transmission, and COVID-19 Mortality: Systematic Review and Meta-Analysis," BMJ, vol. 375, 2021.
[78]. P. H. Edelstein, C. S. Jørgensen, and L. A. Wolf, "... and BinaxNOW Assays for the Detection of Urine and Cerebrospinal Fluid Streptococcus Pneumoniae and Legionella Pneumophila Serogroup 1 Antigen in Patients...," PLoS One, 2020.
[79]. S. Mazamay, J. F. Guégan, N. Diallo, D. Bompangue, E. Bokabo, J. J. Muyembe, and H. Broutin, "An Overview of Bacterial Meningitis Epidemics in Africa from 1928 to 2018 with a Focus on Epidemics 'Outside-the-Belt'," BMC Infectious Diseases, vol. 21, pp. 1-13, 2021.
[80]. J. A. Suaya, M. A. Fletcher, L. Georgalis, A. G. Arguedas, J. M. McLaughlin, G. Ferreira, and T. Verstraeten, "Identification of Streptococcus Pneumoniae in Hospital-Acquired Pneumonia in Adults," Journal of Hospital Infection, vol. 108, pp. 146-157, 2021.
[81]. S. Sharma, J. Acharya, D. A. Caugant, M. R. Banjara, P. Ghimire, and A. Singh, "Detection of Streptococcus Pneumoniae, Neisseria Meningitidis and Haemophilus Influenzae in Culture Negative Cerebrospinal Fluid Samples from Meningitis Patients Using a Multiplex Polymerase Chain Reaction in Nepal," Infectious Disease Reports, vol. 13, no. 1, pp. 173-180, 2021.
[82]. R. Kukla, R. Bolehovska, J. Radocha, L. Pliskova, P. Zak, F. Vrbacky, and H. Zemlickova, "Improved Laboratory Diagnostics of Streptococcus Pneumoniae in Respiratory Tract Samples Through qPCR," New Microbiologica, vol. 43, no. 2, pp. 70-77, 2020.
[83]. Wagner and B. Weinberger, "Vaccines to Prevent Infectious Diseases in the Older Population: Immunological Challenges and Future Perspectives," Frontiers in Immunology, 2020.
[84]. Scelfo, F. Menzella, M. Fontana, G. Ghidoni, C. Galeone, and N. C. Facciolongo, "Pneumonia and Invasive Pneumococcal Diseases: The Role of Pneumococcal Conjugate Vaccine in the Era of Multi-Drug Resistance," Vaccines, vol. 9, no. 5, 420, 2021.
[85]. G. M. Lee, "Preventing Infections in Children and Adults with Asplenia," Hematology 2014, The American Society of Hematology Education Program Book, vol. 2020, no. 1, pp. 328-335.
[86]. F. Micoli, M. R. Romano, F. Carboni, R. Adamo, and F. Berti, "Strengths and Weaknesses of Pneumococcal Conjugate Vaccines," Glycoconjugate Journal, vol. 40, no. 2, pp. 135-148, 2023.
[87]. B. A. Mungall, B. Hoet, J. Nieto Guevara, and L. Soumahoro, "A Systematic Review of Invasive Pneumococcal Disease Vaccine Failures and Breakthrough with Higher-Valency Pneumococcal Conjugate Vaccines in Children," Expert Review of Vaccines, vol. 21, no. 2, pp. 201-214, 2022.
[88]. S. S. Kadri, Y. L. Lai, S. Warner, J. R. Strich, A. Babiker, E. E. Ricotta, and J. Adjemian, "Inappropriate Empirical Antibiotic Therapy for Bloodstream Infections Based on Discordant In-Vitro Susceptibilities: A Retrospective

Cohort Analysis of Prevalence, Predictors, and Mortality Risk in US Hospitals," The Lancet Infectious Diseases, vol. 21, no. 2, pp. 241-251, 2021.
[89]. C. Rhee, S. S. Kadri, J. P. Dekker, R. L. Danner, H. C. Chen, D. Fram, and the CDC Prevention Epicenters Program, "Prevalence of Antibiotic-Resistant Pathogens in Culture-Proven Sepsis and Outcomes Associated with Inadequate and Broad-Spectrum Empiric Antibiotic Use," JAMA Network Open, vol. 3, no. 4, e202899, 2020.
[90]. E. Mühlberg, F. Umstätter, C. Kleist, W. Mier, and P. Uhl, "Renaissance of Vancomycin: Approaches for Breaking Antibiotic Resistance in Multidrug-Resistant Bacteria," Canadian Journal of Microbiology, vol. 66, no. 1, pp. 11-16, 2020.
[91]. K. Bush and P. A. Bradford, "Epidemiology of β-Lactamase-Producing Pathogens," Clinical Microbiology Reviews, 2020.
[92]. Løchen, N. J. Croucher, and R. M. Anderson, "Divergent Serotype Replacement Trends and Increasing Diversity in Pneumococcal Disease in High Income Settings Reduce the Benefit of Expanding Vaccine Valency," Scientific Reports, 2020.
[93]. M. Berman-Rosa, S. O'Donnell, M. Barker, and C. Quach, "Efficacy and Effectiveness of the PCV-10 and PCV-13 Vaccines Against Invasive Pneumococcal Disease," Pediatrics, 2020.
[94]. C. B. Hansen, K. Fuursted, P. Valentiner-Branth, T. Dalby, C. S. Jørgensen, and H. C. Slotved, "Molecular Characterization and Epidemiology of Streptococcus Pneumoniae Serotype 8 in Denmark," BMC Infectious Diseases, vol. 21, no. 1, 421, 2021.
[95]. D. T. Akahoshi and C. L. Bevins, "Flagella at the Host-Microbe Interface: Key Functions Intersect with Redundant Responses," Frontiers in Immunology, 2022.
[96]. N. Zhao, H. Ren, J. Deng, Y. Du, Q. Li, P. Zhou, and T. Qin, "Genotypic and Phenotypic Characteristics of Moraxella Catarrhalis from Patients and Healthy Asymptomatic Participants among Preschool Children," Pathogens, vol. 11, no. 9, 984, 2022.
[97]. J. Vlaeminck, D. Raafat, K. Surmann, L. Timbermont, N. Normann, B. Sellman, and S. Malhotra-Kumar, "Exploring Virulence Factors and Alternative Therapies Against Staphylococcus Aureus Pneumonia," Toxins, vol. 12, no. 11, 721, 2020.
[98]. B. Chang, K. Tamura, H. Fujikura, H. Watanabe, Y. Tanabe, K. Kuronuma, and K. Oishi, "Pneumococcal Meningitis in Adults in 2014–2018 After Introduction of Pediatric 13-Valent Pneumococcal Conjugate Vaccine in Japan," Scientific Reports, vol. 12, no. 1, 3066, 2022.
[99]. M. Masomian, Z. Ahmad, L. Ti Gew, and C. L. Poh, "Development of Next Generation Streptococcus Pneumoniae Vaccines Conferring Broad Protection," Vaccines, 2020.
[100]. P. J. Hotez, "Vaccines Did Not Cause Rachel's Autism: My Journey as a Vaccine Scientist, Pediatrician, and Autism Dad," 2021.
[101]. M. N. van Kassel, G. de Boer, S. A. Teeri, D. Jamrozy, S. D. Bentley, M. C. Brouwer, and M. W. Bijlsma, "Molecular Epidemiology and Mortality of

Group B Streptococcal Meningitis and Infant Sepsis in the Netherlands: A 30-Year Nationwide Surveillance Study," The Lancet Microbe, vol. 2, no. 1, e32, 2021.

[102]. P. O. Narváez, S. Gomez-Duque, J. E. Alarcon, P. C. Ramirez-Valbuena, C. C. Serrano-Mayorga, J. Lozada-Arcinegas, and L. F. Reyes, "Invasive Pneumococcal Disease Burden in Hospitalized Adults in Bogota, Colombia," BMC Infectious Diseases, vol. 21, pp. 1-12, 2021.

[103]. Y. H. Jin, L. Cai, Z. S. Cheng, H. Cheng, T. Deng, Y. P. Fan, and the Zhongnan Hospital of Wuhan University Novel Coronavirus Management and Research Team, "A Rapid Advice Guideline for the Diagnosis and Treatment of 2019 Novel Coronavirus (2019-nCoV) Infected Pneumonia (Standard Version)," Military Medical Research, vol. 7, pp. 1-23, 2020.

[104]. G. B. Nair and M. S. Niederman, "Updates on Community Acquired Pneumonia Management in the ICU," Pharmacology & Therapeutics, 2021.